# Reduction of Normal Flora by Irradiation and Its Effect on the Ability of *Listeria monocytogenes* to Multiply on Ground Turkey Stored at 7°C When Packaged under a Modified Atmosphere†

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## ABSTRACT

*Listeria monocytogenes* did not multiply faster during storage at 7°C on irradiated than on nonirradiated raw ground turkey, and there was a concentration-dependent inhibition of its multiplication by $CO_2$. Ground turkey was gamma irradiated at 5°C to 0, 1.5, and 2.5 kGy and inoculated (~100 CFU/g) after irradiation with a cocktail of *L. monocytogenes* ATCC 7644, 15313, 49594, and 43256. The meat was then packaged in air-permeable pouches or under atmospheres containing 30 or 53% $CO_2$, 19% $O_2$, and 51 or 24% $N_2$ and stored at 7°C for up to 28 days. A dose of 2.5 kGy extended the time for the total plate count (TPC) to reach $10^7$ CFU/g from 4 to 19 days compared to that for nonirradiated turkey in air-permeable pouches. Following a dose of 2.5 kGy at the end of the 28-day study, the TPCs were $10^{6.42}$ and $10^{4.98}$ under 25% and 50% $CO_2$ atmospheres, respectively. Under air, 30% $CO_2$, and 53% $CO_2$ atmospheres, the populations of *L. monocytogenes* after 19 days incubation were $10^{4.89}$, $10^{3.60}$, and $10^{2.67}$ CFU/g. The populations of lactic acid bacteria and anaerobic or facultative bacteria were also reduced by irradiation. Irradiating ground turkey did not decrease its safety when it was contaminated following processing with *L. monocytogenes*.

Various authors have expressed concerns that irradiating food may affect safety by decreasing the competition of indigenous microflora with foodborne pathogens, allowing the unrestricted multiplication of the pathogen without an associated indication of spoilage. Their main concern was that irradiation might so decrease the indigenous population of spoilage bacteria that spores of *Clostridium botulinum*, if present, might germinate and produce botulinal toxin before there were obvious signs of spoilage. Several studies indicate that toxin is not produced any faster in or on irradiated than on nonirradiated foods *(4–6, 16)*.

Licciardello et al. *(10)* noted that *Salmonella* surviving a low dose of radiation on chicken meat did not grow faster than those on unirradiated controls. Matches and Liston *(11)*, in contrast, had observed more rapid growth of *Salmonella* Derby, *Salmonella* Heidelberg, and *Salmonella* Typhimurium on irradiated fish fillets. Tiwari and Maxcy *(17)* observed that the comparative growth of salmonellae, coliforms, and other members of the microflora was similar on irradiated and nonirradiated ground beef. Szczawińska et al. *(15)* inoculated irradiated (0, 1.25, or 2.5 kGy) mechanically deboned chicken with approximately 1,000 CFU/g of *Salmonella* Typhimurium, *Salmonella* Dublin, or *Salmonella* Enteritidis and incubated the samples at 5, 10, or 20°C. The final populations of *Salmonella* Dublin and *Salmonella* Typhimurium, but not that of *Salmonella* Enteritidis, after storage at 10 or 20°C were slightly higher (about 0.5 log) in the samples that had been irradiated than in those that had not been irradiated before inoculation. The results emphasized the need for proper refrigeration.

Growth of *Listeria monocytogenes* (inoculated to either 100 or 10,000 CFU/g) was slow, and spoilage was detectable on poultry carcasses that had been irradiated to 2.5 kGy and stored at 10°C for 7 days *(12)*. Patterson et al. *(13)* irradiated raw and cooked poultry meat inoculated with *L. monocytogenes* to 0, 1.0, and 2.5 kGy and observed that irradiation resulted in significantly increased lag times for this pathogen.

Hashim et al. *(8)* irradiated fresh and frozen chicken to 1.66 to 2.86 kGy and determined the effects of processing on the sensory attributes of both raw and cooked meat. These authors did not find significant effects upon the appearance or taste of cooked breast meat. They found that raw irradiated chicken had higher "fresh chickeny" intensities compared to nonirradiated samples. Cooked irradiated frozen dark meat had more chicken flavor and cooked irradiated refrigerated dark meat was more tender than the controls. Kanatt et al. *(9)* irradiated minced chicken to 2.5 kGy and stored it at 0 to 3°C for up to 4 weeks. The irradiated meat was microbiologically safe and sensorially acceptable up to 4 weeks in the nonfrozen state; the nonirradiated minced chicken had a shelf-life of less than 2 weeks.

The objectives of this study were (i) to test the hypothesis that irradiation pasteurization (≤2.5 kGy) will not significantly influence the ability of *L. monocytogenes* to compete with the residual indigenous microbial flora associated with ground poultry meat during storage at a mild abuse temperature of 7°C, and (ii) to test the hypothesis that an at-

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mosphere enriched with $CO_2$ does not alter the survival and/or multiplication of *L. monocytogenes* during storage.

## MATERIALS AND METHODS

**Concept.** Samples of commercial ground turkey would be irradiated to 0, 1.5, and 2.5 kGy to decrease the population of the indigenous microbial flora. The meat would then be uniformly inoculated with approximately 100 CFU/g of a mixture of *L. monocytogenes* isolates. Thus, the variable would be the effect of the radiation doses on the indigenous microbial flora and not the inoculum of uninjured *L. monocytogenes*. The ground meat would be packaged in 25-g amounts in air-permeable poultry packaging or in modified atmospheres containing oxygen, nitrogen, and 25 or 50% $CO_2$. After packaging, the samples would be stored at a mild abuse temperature of 7°C and the changes in the microbial populations measured during the study.

**Experimental design.** The following experimental variables were used: three replicates; radiation doses: 0, 1.5, and 2.5 kGy; atmosphere: air-permeable packaging, 25:25:50% ($CO_2$, $O_2$, $N_2$), and 50:25:25% ($CO_2$, $O_2$, $N_2$); incubation at 7°C for a maximum of 28 days. Microbiological analyses (total plate count [TPC], *L. monocytogenes*, lactobacilli, facultative anaerobes, and coliforms) and using separate uninoculated samples (sensorial analyses: pH, odor, and appearance) were performed at the times indicated in the results. Due to time and equipment limitations it was necessary to divide the study into three by radiation dose.

Because there might be variations in inocula between the studies described above, a separate study was designed with three replicates: radiation doses of 0, 1.5, and 2.5 kGy before inoculation with *L. monocytogenes*. The meat was packaged as above using air-permeable packaging and analyzed for *L. monocytogenes*.

**Bacterial cultures.** Four isolates of *L. monocytogenes*, ATCC 7644, 15313, 43256, and 49594 (Scott A), were obtained from the American Type Culture Collection (Manassas, Va.). Cultures were maintained at 37°C, and isolated colonies were picked from tryptic soy agar (Difco Laboratories, Detroit, Mich.). Culture identity was confirmed by Gram stains and from reactions on gram-positive identification cards using the Vitek AMS (AutomicrobicSystem, Bio-Mérieux Vitek, Inc., Hazelwood, Mo.) *(1)*. Each isolate was cultured independently at 37°C in 100 ml of tryptic soy broth (Difco) in a baffled 500-ml Erlenmeyer culture flask agitated at 150 rpm on a rotary shaker for 18 h. Equal amounts of each culture were mixed just before use and a dilution prepared to provide an approximate inoculum of 100 CFU/g of meat. Plate counts were performed on each culture as well as on the mixed culture during each experiment. All results are expressed as the logarithm CFU.

**Substrate.** Three lots of commercial ground turkey were purchased at a local grocery store for these studies. The proximate analysis for the meat was moisture 71.3 ± 2.4%, fat 9.8 ± 3.0%, ash 0.96 ± 0.07%, and protein 17.8 ± 0.4%. Approximately 2,100 g of ground meat were required per replicate for each radiation dose. Each sample consisted of 25 g of ground turkey.

**Gas source.** A Smith Standard 180 SCFH Proportional Tri-Gas Blender (Watertown, S.D.) was used to prepare gas mixtures from certified nitrogen, carbon dioxide, and oxygen. The proper settings for the gas mixtures were determined at the start of the study via gas chromatography of each mixture and during the study from each sample.

**Packaging.** Mil-B-131, type I, class I, 5.0-mil (0.127-mm) thick polypropylene-polyethylene-aluminum foil-polyethylene barrier pouches with an $O_2$ transmission of 0.093 cc $m^{-1}$ 24 $h^{-1}$ (Bell Fibre Products, Columbus, Ga.) were used to provide a barrier highly impermeable to gases. In addition, to ensure the presence of a gas envelope over the samples, they were placed in the bottom of a sterile petri dish that was placed within a 12.5- by 12.5- by 1.5-cm polystyrene meat tray. The tray containing the petri dish was placed inside the pouch. The pouches were sterilized before use by gamma irradiation (25 kGy) at ambient temperature.

A microprocessor-controlled Multivac A300 vacuum packager (Kansas City, Mo.) was used to evacuate to 40 mm Hg and to flush with an appropriate gas mixture to 700 mm Hg twice before sealing the pouch. To protect workers from aerosols that might contain *L. monocytogenes*, exhaust gases were passed through a HEPA filter.

**Radiation source, techniques, and dosimetry.** The self-contained gamma-radiation source (Lockheed Georgia Company) has 23 $^{137}CsCl$ pencils placed in an annular array around a 63.5-cm-high stainless-steel cylindrical chamber with a 22.9-cm internal diameter. The source strength at the time of this study was ~117,355 Ci (4.34 PBq) with a dose rate of 0.10 kGy $min^{-1}$. The dose rate was established using National Physical Laboratory (Middlesex, UK) dosimeters. Corrections for source decay were made monthly. Routine dosimetry was performed using 5-mm-diameter alanine dosimeters (Bruker Instruments, Rjeomstettem, Germany), and the free-radical signal was measured using a Bruker EMS 104 EPR Analyzer *(2, 3)*. Variations in radiation dose absorption were minimized by placing small samples within a uniform area of the radiation field, by irradiating them within a polypropylene container (4 mm wall) to absorb Compton electrons, and by using the same geometry for sample irradiation during each study. Samples were maintained at 5 ± 1°C during irradiation through the thermostatically controlled injection, into the top of the irradiation chamber, of the gas phase from liquid nitrogen. The polypropylene container is uniformly perforated to allow for diffusion of the cold nitrogen gas. Sample temperature was monitored continuously during irradiation with thermocouples taped to two samples in the chamber. Based on measurements of dosimeter responses in several experiments, the actual dose was within ±2% of the target dose.

**Head space gas analysis.** Immediately before opening each sample pouch for microbiological analysis, a 0.5-ml sample was withdrawn from the bag with a gas-tight syringe and needle. The gas sample was analyzed by gas chromatography (Gow-Mac series 580; dual thermal conductivity detectors; ambient temperature; He carrier gas, 120 ml/min; using a 0.25-in. stainless steel CTR I column [Alltech Associates, Inc., Deerfield, Ill.]). The CTR I consists of an outer (183 by 0.64 cm) column packed with an activated molecular sieve and an inner (183 by 0.32 cm) column packed with a porous polymer mixture. The gas chromatograph was calibrated with a commercial certified gas mixture consisting of 4.58% methane, 6.99% oxygen, 15.00% carbon dioxide, and 7.04% carbon monoxide (Scotty I-No. 9799, Scott Specialty Gases, Inc., Durham, N.C.).

**Microbiological analysis.** Samples were assayed for CFU by standard pour-plate procedures with serial dilutions in sterile Butterfield's phosphate (0.25 M $KH_2PO_4$ adjusted to pH 7.2 with NaOH). Each sample was diluted 10-fold and homogenized with a Stomacher lab blender (model 400, Tekmar Co., Cincinnati, Ohio) for 90 s and pour plated in triplicate using the appropriate

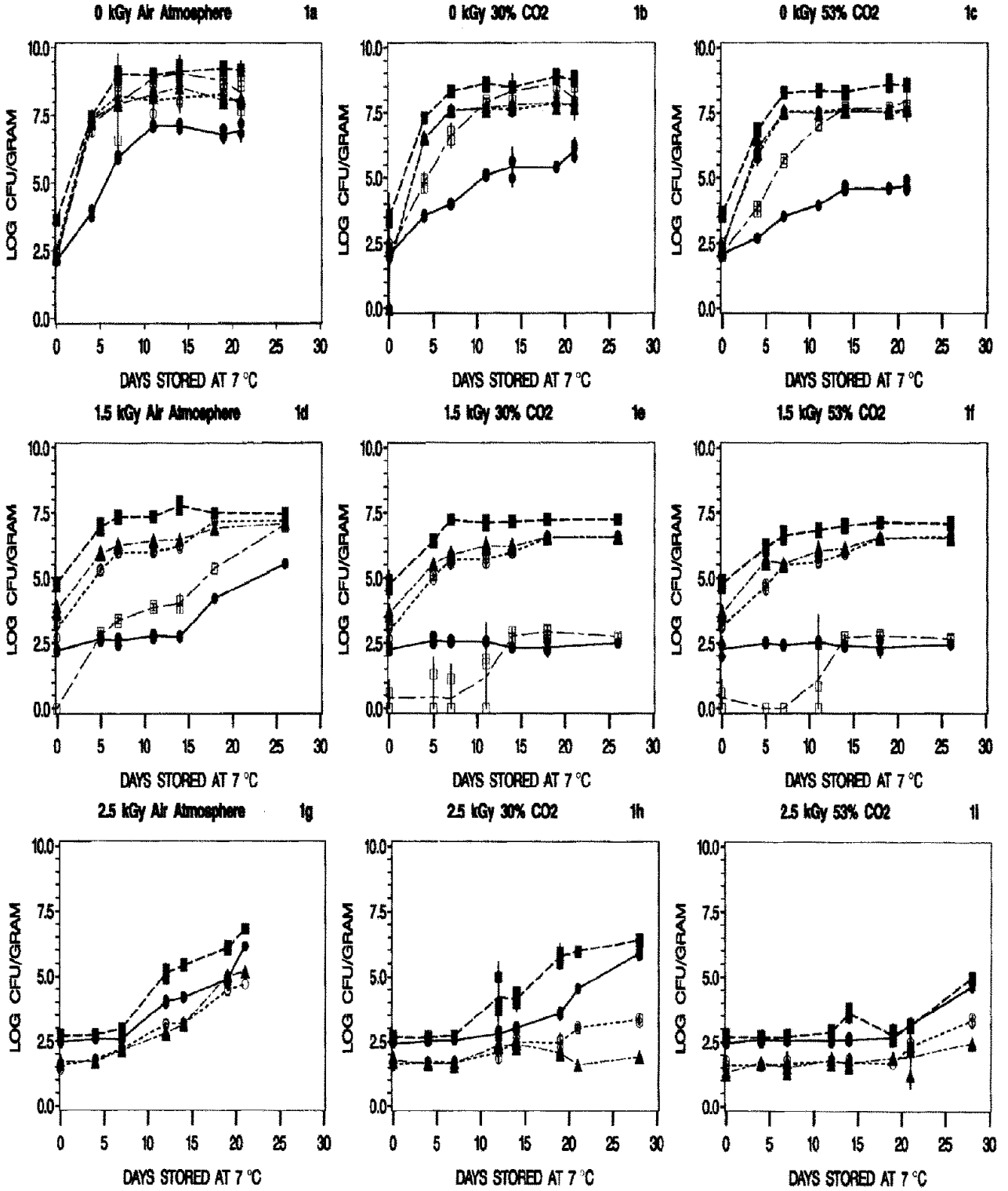


FIGURE 1. *(a) Populations of* L. monocytogenes (●, *solid line), facultative anaerobes* (○, *dotted line), total aerobic* (■, *dashed line), lactic acid bacteria* (▲, *dashes separated by dots), and coliform bacteria* (□, *long dash separated by short dash) on nonirradiated ground turkey under an atmosphere of air over a period of 18 days storage at 7°C. The bars indicate two standard deviations from the means. (b) Populations of* L. monocytogenes (●, *solid line), facultative anaerobes* (○, *dotted line), total aerobic* (■, *dashed line), lactic acid bacteria* (▲, *dashes separated by dots), and coliform bacteria* (□, *long dash separated by short dash) on nonirradiated ground turkey under an atmosphere containing 30% $CO_2$ over a period of 28 days storage at 7°C. The bars indicate two standard deviations from the means. (c) Populations of* L. monocytogenes (●, *solid line), facultative anaerobes* (○, *dotted line), total aerobic* (■, *dashed line), lactic acid bacteria* (▲, *dashes separated by dots), and coliform bacteria* (□, *long dash separated by short dash) on nonirradiated ground turkey under an atmosphere containing 53% $CO_2$ over a period of 28 days storage at 7°C. The bars indicate two standard deviations from the means. (d) Populations of* L. monocytogenes (●, *solid line), facultative anaerobes* (○, *dotted line), total aerobic* (■, *dashed line), lactic acid bacteria* (▲, *dashes separated by dots), and coliform bacteria* (□, *long dash separated by short dash) on ground turkey after irradiation to 1.5 kGy under an atmosphere of air over a period of 28 days storage at 7°C. The bars indicate two standard deviations from the means. (e) Populations of* L. monocytogenes (●, *solid line), facultative anaerobes* (○, *dotted*

medium and conditions of incubation. All samples were incubated at 37°C for 48 h. CFU were counted at a dilution giving 30 to 300 CFU per plate with a New Brunswick Scientific Biotran II automated colony counter. TPCs were obtained with tryptic soy agar (Difco). *L. monocytogenes* CFU/g were obtained using Palcam medium base plus the Palcam antimicrobic supplement (Difco).

The number of CFU/g of lactic acid bacteria was determined using 3M Petrifilm aerobic count plates (3M Microbiology Products, St. Paul, Minn.) with lactobacilli MRS broth (Difco) as the diluent, as suggested by 3M. Multiplication under anaerobic conditions was determined using Petrifilm aerobic count plates with Wilkins Chalgren broth (Oxoid Limited, Basingstoke, England) as the diluent. Lactobacilli and anaerobic Petrifilm plates were incubated anaerobically in a BBL GasPak jar for 48 h at 37°C.

The enteric counts were obtained using Petrifilm *Escherichia coli* count plates (3M Microbiology Products). These were incubated for 24 h at 37°C.

**Statistical analyses.** The means of triplicate plate counts were converted to log CFU/g. To facilitate log analysis, CFU values of zero were assigned a value of 1. The means and population reduction data were analyzed using the general linear model procedure of the SAS statistical package *(7, 14).*

## RESULTS AND DISCUSSION

Because the investigation was divided into three independent studies by radiation dose, with three separate lots of ground meat with different initial indigenous populations, comparisons between doses must be interpreted accordingly. The initial population of *L. monocytogenes* was nearly identical in all three studies, 2.26 ± 0.18 log CFU/g; thus, comparisons can be made between all three studies.

The actual $CO_2$:$O_2$:$N_2$ gas mixtures obtained in the three studies were (29.71 ± 1.38):(19.42 ± 2.03):(50.97 ± 1.18) and (53.25 ± 3.05):(22.43 ± 1.51):(24.32 ± 3.18), respectively. Evidence for a significant regression of $CO_2$ concentration over time during the three studies was not found.

The aerobic, facultatively anaerobic, lactic, and coliform bacterial counts in nonirradiated samples packaged in air-permeable pouches followed a very similar pattern (Fig. 1a). Starting from a TPC of $10^{3.65}$ CFU/g, these populations exceeded $10^7$ CFU/g within 4 days. By 7 days the samples were slimy, off-color, and had a sour-putrid odor. The aerobic, facultative anaerobic, lactic, and coliform bacterial counts in nonirradiated samples packaged in modified atmospheres containing either 30 or 53% $CO_2$ followed a very similar pattern as the populations in the air-permeable packaging; however, there was inhibition of the multiplication of coliform bacteria that was $CO_2$ concentration dependent (Fig. 1b and 1c). The meat gradually turned gray under $CO_2$ over 11 days; however, a spoilage odor was not noticed until 19 days. *L. monocytogenes* populations on nonirradiated ground turkey obtained a maximum population at about 11 days under all three atmospheres. However, the multiplication of *Listeria* was less under $CO_2$ than under air.

The aerobic, facultatively anaerobic, and lactic acid bacteria in samples that were irradiated to an absorbed dose of 1.5 kGy multiplied rapidly even under $CO_2$ atmospheres; however, the rates were reduced under an atmosphere of 53% $CO_2$ (Fig. 1d through 1f). The TPC of the ground turkey decreased from $10^{5.13}$ to $10^{4.77}$ CFU/g when it was irradiated to an absorbed dose of 1.5 kGy. The initial indigenous population of coliforms was reduced to a nondetectable level by irradiation; however, in air-permeable packaging the population rapidly increased during storage. The presence of either 30 or 53% $CO_2$ inhibited the multiplication of coliform bacteria and extended the lag phase (Fig. 1d through 1f). Though the *L. monocytogenes* inoculum was not irradiated, the lag phase was extended and its multiplication was reduced on ground turkey with little or no multiplication of the inoculum occurring under $CO_2$ atmospheres. The cells, however, remained viable as demonstrated by the plate counts. All of the meat samples took on a gray appearance by 5 days; however, a spoilage odor was not detected until 26 days.

The aerobic, facultative anaerobic, and lactic acid bacterial populations were reduced by a radiation dose of 2.5 kGy, and coliform bacteria were eliminated (Fig. 1a, 1d, and 1g). In air-permeable packaging the lag phases were extended to about 7 days; however, later these populations

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line), total aerobic (■, *dashed line), lactic acid bacteria (▲, dashes separated by dots), and coliform bacteria (□, long dash separated by short dash) on ground turkey after irradiation to 1.5 kGy under an atmosphere containing 30% $CO_2$ over a period of 28 days storage at 7°C. The bars indicate two standard deviations from the means. (f) Populations of* L. monocytogenes *(●, solid line), facultative anaerobes (○, dotted line), total aerobic (■, dashed line), lactic acid bacteria (▲, dashes separated by dots), and coliform bacteria (□, long dash separated by short dash) on ground turkey after irradiation to 1.5 kGy under an atmosphere containing 53% $CO_2$ over a period of 28 days storage at 7°C. The bars indicate two standard deviations from the means. (g) Populations of* L. monocytogenes *(●, solid line), facultative anaerobes (○, dotted line), total aerobic (■, dashed line), lactic acid bacteria (▲, dashes separated by dots), and coliform bacteria (□, long dash separated by short dash) on ground turkey after irradiation to 2.5 kGy under an atmosphere of air over a period of 28 days storage at 7°C. The bars indicate two standard deviations from the means. (h) Populations of* L. monocytogenes *(●, solid line), facultative anaerobes (○, dotted line), total aerobic (■, dashed line), lactic acid bacteria (▲, dashes separated by dots), and coliform bacteria (□, long dash separated by short dash) on ground turkey after irradiation to 2.5 kGy under an atmosphere containing 30% $CO_2$ over a period of 28 days storage at 7°C. The bars indicate two standard deviations from the means. (i) Populations of* L. monocytogenes *(●, solid line), facultative anaerobes (○, dotted line), total aerobic (■, dashed line), lactic acid bacteria (□, long-dashed lines), and coliform bacteria (□, long dash separated by short dash) on ground turkey after irradiation to 2.5 kGy under an atmosphere containing 53% $CO_2$ over a period of 28 days storage at 7°C. The bars indicate two standard deviations from the means.*

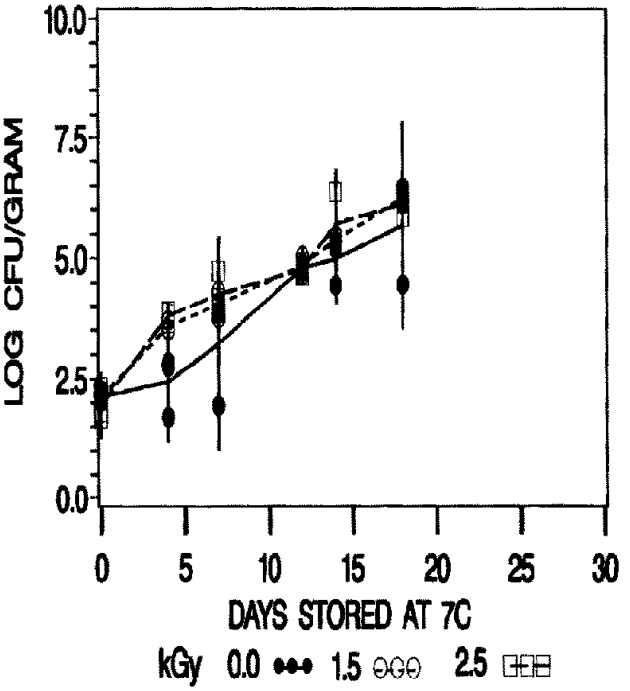


FIGURE 2. *Populations of* L. monocytogenes *on irradiated (0, 1.5, and 2.5 kGy) ground turkey in air-permeable packaging over a period of 18 days storage at 7°C.*

increased rapidly (Fig. 1g). The population of *L. monocytogenes* increased after a lag phase of about 7 days on ground turkey in air-permeable packaging. The modified atmosphere packaging containing $CO_2$ inhibited the multiplication of all of the cultures (Fig. 1h and 1i). All samples turned gray by the 12th day, but the meat never developed a significant off odor.

The effects of irradiation on the TPC (Fig. 1a, 1d, and 1g) are apparent. The TPC of the meat decreased from $10^{3.75}$ to $10^{2.70}$ CFU/g when irradiated to a dose of 2.5 kGy. Irradiation at 2.5 kGy increased the lag phase and the time required to reach a spoilage level of $10^7$ CFU/g from 4 to 19 days. Following a dose of 2.5 kGy at the end of the 28-day study, the TPCs were $10^{6.42}$ and $10^{4.98}$ under 30 and 53% $CO_2$ atmospheres, respectively.

The responses of the indigenous lactic acid bacteria were only slightly inhibited by the presence of $CO_2$ in non-irradiated samples and in those that received a dose of 1.5 kGy (Fig. 1b, 1c, 1e, 1f, 1h, and 1i). Following a radiation dose of 2.5 kGy the lactic acid bacteria multiplied only in air-permeable packaging (Fig. 1h and 1i). Because surviving lactic acid bacteria were easily countable, these results indicate that the $CO_2$ prevented these injured bacteria from recovering and initiating multiplication.

The results of the three independent studies at doses of 0, 1.5, and 2.5 kGy could mean that the multiplication of *L. monocytogenes* was inhibited when placed on irradiated turkey. However, when three batches of meat were irradiated to these same doses and then all inoculated with the same culture there was no significant difference in the multiplication of *L. monocytogenes* (Fig. 2).

*L. monocytogenes* did not multiply at a greater rate on meat that had been treated by irradiation (Fig. 1a through 1i). Thus, the initial hypothesis that irradiation pasteurization (≤2.5 kGy) does not significantly alter the ability of *L. monocytogenes* to compete with the residual indigenous microbial flora associated with ground poultry meat during storage at a mild abuse temperature of 7°C is accepted. The hypothesis that an atmosphere enriched with $CO_2$ does not alter the survival and/or multiplication of *L. monocytogenes* during storage is rejected. Under air, 30% $CO_2$, and 53% $CO_2$ atmospheres, the populations of *L. monocytogenes* after 19 days incubation were $10^{4.89}$, $10^{3.60}$, and $10^{2.67}$ CFU/g.

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